

Practical measurement of total SO₂ in wine

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What are the strengths and weaknesses of the most common methods of SO₂ measurement and what are the minimum quality assurance steps needed to ensure accurate results?

INTRODUCTION

Sulfur dioxide (SO₂) is a preservative widely used in foods and beverages. In wine, SO₂ occurs naturally (generated by yeast) and is a very common additive. Due to the importance of its antioxidant and antimicrobial functions in wine, SO₂ is one of the most commonly analysed wine components and its levels are carefully controlled to ensure effective performance without negative sensory impacts. It is also one of the most commonly regulated wine parameters around the world. As such its accurate and reproducible measurement in wine is of prime importance both in the production of sound and stable wine and to ensure the free and efficient movement of wine between markets.

Given its importance, there is a significant need for standardisation and quality assurance practices when analysing SO₂ to ensure comparable results between laboratories. This article summarises the strengths and weaknesses of the most common methods and the minimum quality assurance steps that should be conducted. Rather than define in detail the steps in each method (which are readily available in a number of resources) it instead discusses the practical considerations, which are not always covered in references.

SULFUR DIOXIDE PATHWAYS TO WINE

Sulfur dioxide can be added at many points during the wine production process. It is not uncommon for the salt potassium metabisulfite (KMBS, or sometimes PMS), which forms SO₂ on dissolution, to be added to grapes in harvest bins immediately after picking. Once at the crusher SO₂ is again commonly added, either as KMBS or liquid SO₂, to prevent oxidation and inhibit unwanted microbial activity.

Once primary fermentation has begun, SO₂ is produced by wine yeast. The amount of SO₂ released depends on the yeast strain and

fermentation conditions, and can range from a few milligrams per litre up to 100mg/L. Sulfur dioxide is not usually added during primary or secondary fermentation due to its potential to inhibit these processes.

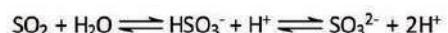
Following primary and secondary fermentation, SO₂ can be added to wine either as KMBS or as liquid SO₂ to control microbial activity and to limit oxidation. During wine storage and maturation, particularly in barrel, SO₂ levels are carefully monitored to ensure that sufficient SO₂ is present. Changes in levels of SO₂ can also be an indicator of unwanted oxidative or microbial activity and, therefore, a trigger for other interventions.

Finally, SO₂ levels are usually adjusted immediately before a wine is packaged to ensure that a sufficient amount is present to ensure adequate protection against oxidation or microbial activity for its foreseeable shelf life. The final levels of SO₂ present in wine from all of these inputs are generally well below the typical regulatory limits found in markets around the world and significantly lower than those found in many other packaged foods. High levels of SO₂ in wine are not generally considered favourable as they can have a negative sensory impact.

Once a wine has been packaged, SO₂ performs its functions in a sacrificial manner, and levels decrease over time. The speed of the decrease is a function of factors including wine composition, the amount of oxygen introduced at the time of packaging, characteristics of the package and closure, and the environmental conditions under which the wine is stored.

FORMS OF SO₂ PRESENT IN WINE

In aqueous solutions such as wine, SO₂ exists as a complex mix of related compounds linked by equilibria. It should be noted that all the different forms in which SO₂ can be added (liquid, gas or salt) equilibrate to the



Molecular Bisulfite Sulfite

Figure 1. The equilibria that exist between SO₂ and water.

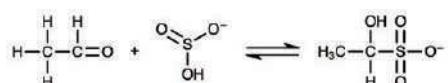


Figure 2. The equilibria that exist between acetaldehyde and bisulfite to form bound SO₂.

same forms in solution. That is, it is not possible in wine to differentiate if the SO₂ present has been naturally generated by yeast, added in the form of liquid SO₂ or as the salt KMBS.

In wine, the primary equilibrium that occurs is between SO₂ and water. This leads to three major forms being present (Figure 1).

Of these three forms, at wine pH, between 92% and 99% is present in the bisulfite form. The proportions of the three species will, however, shift with changes in wine pH and other conditions. The molecular form, usually present in relatively small amounts, is the primary form responsible for the antimicrobial activity of SO₂. The bisulfite form has an important role in the antioxidant properties of SO₂, while the sulfite form is present only in vanishingly small quantities.

SO₂ is also involved in chemical equilibria with other wine components. The most important of these equilibria is that with acetaldehyde (Figure 2), however, bisulfite will also bind weakly to other carbonyls, phenolics, colour compounds and sugars. When SO₂ is bound strongly to wine components in this way, it is generally considered to be unavailable for antimicrobial and antioxidant activities and is referred to as bound SO₂. The unbound component is referred to as free SO₂ and in wine this consists of molecular SO₂ and bisulfite in equilibrium, as described above. The addition of the free and bound quantities of SO₂ is referred to as the total SO₂ of the wine:

$$\text{free SO}_2 + \text{bound SO}_2 = \text{total SO}_2$$

The bound component is usually significantly greater than the free component and, in practise, SO₂ tends to be added to a wine to achieve a given free SO₂ level as this is the active constituent. The proportion of free SO₂ will, however, change over time. Given the relatively complex reactions involved, for practical reasons SO₂ regulatory requirements tend to be phrased in terms of total SO₂, disregarding the differing forms in which it may be present.

METHODS FOR MEASURING TOTAL SULFUR DIOXIDE

There are three generally accepted methods for the determination of total SO₂:

Aeration/oxidation

The most common method used for the determination of total SO₂ is the aeration/oxidation method (AO), which is sometimes known as the Monier/Williams or Rankine/Pocock method, and is the basis of one of the approved OIV methods (OIV-MA-AS323-04A). Essentially, the analysis involves heating an acidified sample of wine to liberate the SO₂ in the molecular form. A stream of air is then used to carry the liberated SO₂ to a reservoir of hydrogen peroxide where it is oxidised to form sulfuric acid. The generated sulfuric acid is then titrated against dilute sodium hydroxide to determine the amount of acid formed and, hence, the original quantity of SO₂ in the wine.

As this method liberates the SO₂ from the sample matrix, it is relatively robust in terms of interferences from other wine components. The equipment is, however, fairly specialised, and reagents and gas flow rates used need to be carefully maintained. Each sample also takes around 20 minutes to analyse and requires constant attention from a trained technician. The method is, however, very robust and repeatable. A detailed description is provided in Illand *et al.* 2004.

Automated flow techniques

These techniques use either flow injection analysis or segmented flow instruments to automate the determination of total SO₂. Essentially, they work by passing a heated, acidified wine solution past a gas permeable membrane. This liberates the SO₂ which

passes through the membrane into a buffered carrier solution and then reacts with a SO₂-sensitive dye such as pararosaniline. The interaction of the dye and the SO₂ forms a coloured solution, with the intensity of the colour then measured spectrophotometrically. This response is compared with a standard curve to indicate the amount of total SO₂ present in the original wine.

While capable of providing results comparable to AO, these techniques require relatively expensive equipment and are best suited to facilities that run large numbers of samples each day. They also tend to require a high degree of maintenance by trained technicians.

Iodic titration

Iodic titration (described in the OIV method OIV-MA-AS323-04B), also commonly known as the Ripper method, involves the titrating of a wine sample against a standardised iodine solution in the presence of starch (or vitex indicator) after converting the SO₂ present to the bisulfite form by the addition of sodium hydroxide. The bisulfite reduces the molecular iodine to iodate until all the bisulfite present has been exhausted, at which point the solution turns blue due to the interaction of the free iodine with the starch indicator. The total SO₂ is then calculated by the amount of iodine required to reach the blue colour. Some analytical equipment suppliers have automated this procedure using autotitrators and various indicator electrodes.

While much quicker and simpler than the AO methodology, iodide titration suffers from significant interferences from wine components. Red wines in particular are known to give erroneously high results due to the reaction of some of the colour compounds. The presence of any ascorbic or erythorbic acid also quantitatively reacts with iodine and this method cannot be used in wine that may contain these preservatives, unless they have first been quantified. It is generally acknowledged that iodide titration methods give a less accurate determination of total SO₂ than AO methods for these reasons.

Other methods

There are several other analytical methods available in kit form

which tend to rely on the use of coloured dye in buffered wine solutions and measurement in either spectrophotometers or sequential analysers. One high throughput method using a specific sequential analyser has recently been validated and is described in Porter *et al.* (2017). There are also several electrochemical methods. These, however, tend to be low throughput and require specialised equipment and training. They, therefore, offer little or no advantage over the other methods described for total SO₂ analysis.

SAMPLE HANDLING AND MANAGEMENT

SO₂ is a relatively volatile compound, which means that sample handling and management are critical to ensure accurate and precise results. The following are the main sample handling considerations:

- Samples should not be opened until immediately before analysis to avoid loss of SO₂ through volatility or oxidation.
- If a sample must be decanted, it should be done very carefully to avoid any aeration into a glass container which can be sealed with no air space under the closure.
- If decanted samples must be stored, they should be refrigerated. Freezing samples is not recommended.
- Refrigerated samples should be allowed to equilibrate to 20°C in sealed vessels before analysis.
- During transfer and pipetting of samples great care should be taken to avoid aeration of the sample.

BEST PRACTICE QUALITY ASSURANCE

It is critical that quality assurance steps are followed during analysis of total SO₂ to ensure consistency of results and comparability of results between laboratories. The steps outlined below are considered the minimum necessary to ensure precise results. It should be noted that they do not negate the need for a vigorous validation process when a method is first introduced to a laboratory or when significant changes are made to equipment, reagents or procedures.

Control samples

Every first and tenth sample should be a matrix-matched wine of known total SO₂. In the case of fewer than 10 samples being run on a given day, the

sample run should be bracketed by control samples. The control wine can be any wine of known total SO₂, either through analysis by another trusted laboratory, or more commonly, by comparative overlap with a previous control wine.

It is generally acceptable to use a cask (boxed) wine as a control wine as this allows a greater interval before the need to validate a new control sample. If a cask wine is used, it is important that it is stored in a refrigerator and great care is taken in decanting the wine from the cask to avoid oxidation and loss of total SO₂.

The results for the control samples should form the basis of a control chart, with the variation from the mean not exceeding 5%. If a cask wine is used as a control, allowance must be made for the slow degradation of total SO₂ due to oxygen ingress across the cask membrane. This is most easily achieved by using a moving average for the central tendency line of the control chart.

If automated flow techniques are in use, it is recommended that two separate control wines (red and white) are used to ensure that there is no difference in matrix dependency. This is not generally considered necessary for AO methods.

Duplicate samples

Duplicate samples can also be run, but the use of duplicates should not replace the use of a control wine,

as quite often the duplicates will not necessarily highlight issues with equipment or reagents.

Known standard

On a weekly basis, a freshly made standard 100mg/L SO₂ solution should be analysed. The recovery from the analysis of this solution should be within ±5%. Details on the preparation of this standard can be found in Iland *et al.* 2004.

Volatile acidity interference check

The presence of high levels of acetic acid is one of the few possible interferents in the AO method. It is negated by the use of an appropriate condenser between the sample and the hydrogen peroxide solution, as well as careful control of the carrier air flow rate, which should be regularly checked to ensure a 1L/min flow. To check the effectiveness of the equipment, a 1g/L acetic acid solution should be analysed at least once a week with no more than 1mg/L of calculated total SO₂ apparent. This procedure is not applicable for analysis by iodide titration.

CONCLUSION

Sulfur dioxide is an important component of wine due to its antimicrobial and antioxidant properties. There are several methods that can be used to measure total SO₂, however, the one most easily implemented in small to medium wine laboratories and is free of significant interferences is aeration/oxidation. Automated flow methods tend to be more suited to high

throughput specialist wine laboratories, while iodide titration suffers from a range of interferences which can lead to erroneously high results.

Independent of the method used, the volatile nature of SO₂ and its sensitivity to oxidation mean that careful sample handling and rigorous quality assurance procedures are required to achieve accurate and precise results. With this in mind, the importance of appropriate validation of all laboratory methods (rather than simply following a published method) cannot be overstated.

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